iForest
Biogeosciences and Forestry

Research Article
doi: 10.3832/ifor3180-013
vol. 13, pp. 251-260

Decline in commercial pine nut and kernel yield in Mediterranean stone pine (*Pinus pinea* L.) in Spain

Rafael Calama [(1-2)], Javier Gordo [(3)], Sven Mutke [(1-2)], Mar Conde [(1)], Guillermo Madrigal [(1-2)], Enrique Garriga [(4)], María José Arias [(5)], Míriam Piqué [(6)], Rodrigo Gandía [(7)], Gregorio Montero [(1-2)], Marta Pardos [(1-2)]

Cones of the Mediterranean stone pine (*Pinus pinea* L.) constitute one of the most relevant non-wood forest products collected in the Mediterranean forests, providing high value edible kernels. In the last years it has been observed a severe decline in the kernel-per-cone yield (kg of kernels obtained from a kg of fresh cones) through the whole area of the species. This decline has been associated with both ongoing climate change and the recent expansion over the Mediterranean Basin of the Western Conifer Seed Bug, *Leptoglossus occidentalis* Heideman, an exotic pest which predates seeds of conifer species. In the present work we aimed to confirm and quantify the impact of this recent decline on pine nut and kernel production, identify the main factors provoking this reduction, and give evidence over causality by a potential biotic agent. We analysed recent and historical series of pine nut and kernel production obtained in the four main regions where *Pinus pinea* occurs in Spain. Our results showed a significant drop in the final kernel-per-cone yield on three of the four regions analysed, reaching reductions over 50% in the most affected areas. We observed that this reduction is mainly associated with a significant and generalised drop in the kernel-per-nut yield (kg of kernels per kg of pine nuts in shell), triggered by an increment in the rate of damaged pine nuts and, to a lesser extent, a reduction in the number of pine nuts per cone. The prevalence of this reduction on kernel-per-cone yield over different years and provenances with contrasting climate reinforces the hypothesis of the implication of a biotic factor which can be aggravated on extreme drought years.

Keywords: *Leptoglossus occidentalis*, Kernel-per-Cone Yield, Cones, Exotic Pest, Seed Predation

(1) INIA-Forest Research Centre, Dept. of Forest Management and Dynamics, Ctra A Coruña km 7.5, 28040 Madrid (Spain); (2) iuFOR, Sustainable Forest Management Research Institute, UVa-INIA (Spain); (3) INIA-Forest Research Centre, Dept. of Forest Products, Ctra A Coruña km 7.5, 28040 Madrid (Spain); (4) Dept. of Forest Products, INIA-CIFOR, Ctra A Coruña km 7.5, 28040 Madrid (Spain); (5) Agencia de Medio Ambiente y Agua de Andalucía, c/ Johan G. Gutenberg, 41092 Sevilla (Spain); (6) Joint Research Unit CTFC- AGROTECNIO, 25280 Solsona (Spain); (7) Servicio Territorial de Medio Ambiente de Ávila, Pasaje del Císter 1, 05071 Ávila (Spain)

@ Rafael Calama (rcalama@inia.es)

Received: Jun 28, 2019 - Accepted: Apr 29, 2020

Citation: Calama R, Gordo J, Mutke S, Conde M, Madrigal G, Garriga E, Arias MJ, Piqué M, Gandía R, Montero G, Pardos M (2020). Decline in commercial pine nut and kernel yield in Mediterranean stone pine (*Pinus pinea* L.) in Spain. iForest 13: 251-260. - doi: 10.3832/ifor3180-013 [online 2020-07-03]

Communicated by: Massimo Faccoli

Introduction

Cones of the Mediterranean stone pine (*Pinus pinea* L.) constitute one of the most important non-wood forest products collected in the Mediterranean Basin. Each cone renders up to 100-120 seeds, called "pine nuts", whose small edible, ivory-white kernels, have been appreciated for their flavour since ancient times. In addition, they have excellent dietetic values with high content in proteins (35%) and minerals (phosphorus, iron, zinc, magnesium) but a low content in saturated fatty acids (Evaristo et al. 2010, Salas-Salvadó et al. 2011). The Mediterranean pine nut, as one of the most important wild edible nuts collected in the world, is mainly produced in Spain, Portugal, Turkey and Italy (INC 2012). Cones are collected when they are still closed, during fall and winter seasons. Once in the industry, collected cones can be processed immediately by boiling into water to open or can be stored outdoors or sheltered in the factory and air dried up until cone dehiscence, which takes place in May-June. The subsequent industrial process includes cone opening, separation of woody parts from the pine nuts which are still in shell, storage and final shelling of pine nuts to obtain the kernels. Cones yield in weight corresponds to 15-20% seeds and only 3-4% kernels, giving 1 kg of kernels per 25-30 kg of fresh cones, varying widely between years and regions (Gordo et al. 1999, Montero et al. 2004).

This low percentage of edible kernels together with the limited supply associated with wild-collected food, might explain the high retail prices for kernels, exceeding 60-70 € kg^{-1}. Due to the high price paid, many stone pine forest owners have orientated the management of the species towards optimizing cone rather than timber production (Pasalodos-Tato et al. 2016), backed up by an extensive scientific knowledge on optimal management (Freire et al. 2016), tree breeding (Mutke et al. 2005) and domestication based on agronomy techniques (Guadaño et al. 2016).

Several sources of uncertainty condition the commercial and industrial process of cone transformation. The first is the lack of sustained cone supply from forests, linked to the large interannual variability in cone production, this variability reaching more than 100-fold in interior Spain (Calama et al. 2016a). The masting habit of the species is largely triggered by climate events (mainly rainfall during key phases of cone development) affecting strobilus induction in the year prior to female flower emergence, *i.e.*, three years before cone collection (Mutke et al. 2005, Calama et al. 2016a). Drought or extreme winter frosts during the three-year process of cone development can cause conelet losses, reducing the final crop of cones. In addition, resource depletion during a bumper crop inhibits the induction of the next female conelet cohort (Mutke et al. 2005).

A second source of uncertainty is the potential effect of cone pests. Two native insects are the main agents of biotic damage in immature *P. pinea* cones throughout the distribution area of the species (Bracalini et al. 2013): the pine cone moth *Dioryctria mendacella* Stgr. and the weevil *Pissodes validirostris* Gyll. Larvae of both species mine feeding galleries in the cones, destroying their commercial value, with recent studies pointing to a major incidence of *D. mendacella* that can affect 20-30% of the total annual cone production (Calama et al. 2017a).

The last issue of concern is related with pine nut yield in cones. The raw material entering the cone processing industry are the fresh closed green cones. Industry processors aim to obtain the largest "kernel-per-cone" yield, defined as kg of kernels obtained per kg of fresh cones. This kernel-per-cone yield is the product of two intermediate yields, the "nuts-per-cone yield", defined as kg of shelled pine nuts obtained from a kg of fresh cones, and the "kernel-per-nut yield" defined as kg of kernels obtained per kg of pine nuts.

The nuts-per-cone yield is largely linked to parameters such as the average number of pine nuts per cone or per kg of fresh cones and mean size (weight) of pine nuts (Morales 2009). These parameters are positively correlated with the average weight of a single cone (Mutke et al. 2007), which can roughly vary between 100-500 g and is largely controlled by the rainfall amount during the last year of cone maturation (Mutke et al. 2007, Guadaño et al. 2016, Loewe-Muñoz et al. 2019). As a consequence, nuts-per-cone yield has historically shown large between-year variability, ranging from 0.08 to 0.20, associated with annual climate conditions.

As regards kernel-per-nut yield, up to 2010 it was relatively constant, with values ranging from 0.20-0.24. Interannual variability was mainly associated with the rate of damaged or empty pine nuts which accounted for 10%-20% of the pine nuts of the cone (Montero et al. 2004, Morales 2009). This rate of empty pine nuts is also negatively correlated with the average cone weight and was found to be higher after dry springs with smaller cones (Mutke et al. 2007, Morales 2009).

Since 2012, the alarm has been raised by forest owners, cone pickers and processing industries, that production had dropped off drastically in all cone-producing countries (Sousa et al. 2012, Bracalini et al. 2013, Ozçankaya et al. 2013, Calama et al. 2015). In the field, cone pickers have reported a dramatically high number of dry unripe conelets in their first and second year of development, and processing industries have observed a significant drop in kernel-per-cone yield, derived from percentages of damaged pine nuts (empty or containing only withered remains of the kernel) of over 50% (Mutke et al. 2017). The rapid, coincident and generalised increase in both phenomena, aborted conelets and damaged seeds, has given rise to the term "Dry Cone Syndrome" (DCS), suggesting a possible common agent (Tiberi 2007). However, despite the economic incidence of DCS, very few data from forests and factories have been published that allow the quantification of its incidence (Mutke et al. 2017).

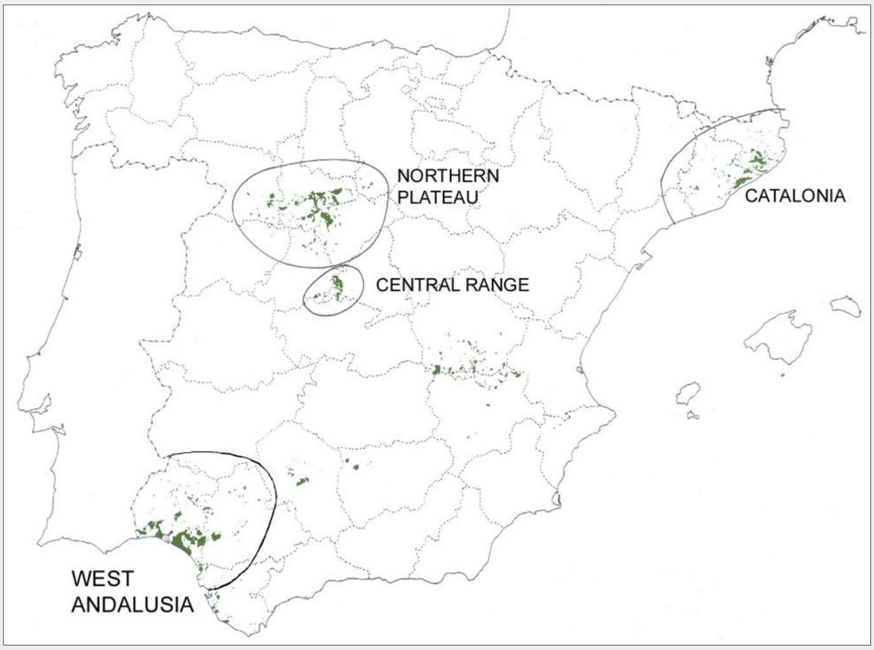

Fig. 1 - The four studied regions in Spain.

With respect to the potential causes of DCS, ongoing climate change and increased prevalence of cone pests were highlighted as putative causes (Sousa et al. 2012, Parlak et al. 2013, Mutke et al. 2017). The temporal coincidence between the first local occurrences of the DCS and the fast spread of the Western Conifer Seed Bug, *Leptoglossus occidentalis*, in Europe (Lesieur et al. 2018) pointed to this exotic insect as a potential cause of the damage. In its native western North America, this bug predates on numerous conifer species, producing the same kind of damage as described in Mediterranean stone pine: aborted conelets, and damaged kernels within apparently normal cones and seeds (Strong et al. 2001, Bates et al. 2002, Lesieur et al. 2014, Farinha et al. 2017). Recent studies using experimental exclusion cages and artificial feeding essays (Ponce et al. 2017, Farinha et al. 2018a), as well as the close similarity between seed damage positively attributable to *L. occidentalis* (from artificial feeding experiments) and the damage observed in the field (Calama et al. 2016b) confirm the implication of biotic agents as the main agent provoking the observed reduction in kernel-per-cone yield associated with DCS. However, due to the absence of an efficient method for monitoring and quantifying the presence of *L. occidentalis* in forest stands (Bates & Borden 2005) it is not possible to identify a direct relationship between the amount of damaged seed and the population size of the insect, thus, caution should be exercised when interpreting the main potential cause to be the insect.

The main objective of this study was to quantify the real impact of the recent decline in kernel-per-cone yield in *Pinus pinea* forests of different Spanish regions. A second objective was to identify which of the two intermediate yields: nuts-per-cone yield or kernel-per-nut yield, has the greatest influence over the final kernel-per-cone yield, evaluating whether there has been a temporal shift in the relative importance of each one. A third objective focused on analysing the temporal evolution of the different covariates governing intermediate yields. A final objective aimed to identify whether the observed recent decline is explained by annual climate fluctuations or can be linked with other causes (*e.g.*, biotic agent), by analysing the relation between the cone weight and the nut content covariates.

In this approach, we compared data from recent cone crop surveys (since 2012) with data from historical crop surveys (prior to the arrival of *L. occidentalis*). Our main hypothesis is that a generalized and not previously observed decrease in kernel-per-cone yield has occurred, which can be associated with other factors apart from annual climate events. Under this hypothesis we would expect that in comparison with historical surveys, recent surveys will show: (i) a more significant decrease in kernel-per-

Tab. 1 - Cone, nut and kernel variables and ratios.

Variable	Unit	Name	Formula	Description
SFW	g	Fresh weight of the set of cones	-	varies with water content (H%)
SCN	-	Number of cones in the set	-	up to 5 cones
CFW	g	Cone fresh weight	CFW = SFW / SCN	Mean weight of one cone
SDW	g	Set dry weight	-	oven-dried (1 week, 45 °C)
CDW	g	Cone dry weight	CDW = SDW / SCN	mean dry weight of one cone
H%	%	Relative Humidity	H% = 1 - (SDW / SFW)	decreases with harvest date
CFW*	g	Standardized cone fresh weight	CFW* = CDW / 63%	mean cone weight at 37 % humidity (November harvest)
NPS	-	Number of pine nuts in the set	-	excl. aborted seeds (< 4 mm)
NPC	-	Number of pine nuts per cone	NPC = NPS / SCN	-
NP_kg	kg^{-1}	Number of pine nuts per kg of cones	NP_kg = NPC / CFW*	-
WPS	g	Weight of pine nut sample	-	in shell
NP_SS	-	Number of pine nuts in subsample	-	up to 50 pine nuts
WP_SS	g	Weight of pine nuts in the subsample	-	-
WP	g	Pine nut mean weight in shell	WP = WPS / NPS	Mean weight of one pine nut
NK_SS	-	Number of kernels in the subsample	-	excl. empty or damaged seeds
D	-	Rate of damaged or empty nuts	1- (NK_SS / NP_SS)	-
WK_SS	g	Weight of kernels from subsample	-	-
WK	g	Kernel mean weight	W= WK_SS / NK_SS	Mean weight of one kernel
NCY	-	Nut-per-cone yield	NCY = NP_kg · WP	weight of inshell pine nuts obtained from one kg of cones
KNY	-	Kernel-per-nut yield	KNY = WK_SS / WP_SS	weight of kernels obtained from one kg of inshell pine nuts
KCY	-	Kernel-per-cone yield	KCY = NCY · KNY	weight of kernels obtained from one kg of cones

nut yield and a larger influence of this component over the final kernel-per-cone yield; (ii) a greater influence of the rate of damaged pine nuts in both kernel-per-nut and kernel-per-cone yields; (iii) a significant and maintained increase in the rate of damaged or empty pine nuts, irrespective of interannual changes in climate conditions and average cone weight.

Materials and methods

Studied regions

The studied regions include the four most important provenance regions for the species in Spain (Prada et al. 1997 – Fig. 1): Catalonia, South West Andalusia, Northern Plateau and the Central Range. These four regions differ considerably in terms of ecological conditions and traditional management, as summarised in Tab. S1 (Supplementary material).

Cone collection

Historical surveys

Between 1992 and 2000 INIA-CIFOR installed a network of permanent plots in pure even-aged stands of *Pinus pinea* in the four studied regions. The plots were circular, with variable radius in order to include 20 trees. Plot location aimed to cover the whole range of site quality, stand stocking and age classes identified in each region. Mature cones were collected every autumn from the five trees closest to the centre of the plot, counting and weighing healthy and damaged cones (*Dioryctria mendacella* and *Pissodes validirostris*). A subsample of five cones per tree, without signs of damage, were randomly selected and sent to the laboratory for further analysis related to pine nut and kernel yield and quality. While annual cone collection in these experimental plots is still ongoing (*e.g.*, in the case of the Northern Plateau, there is now a temporal series covering more than 20 years of fruit production), nut and kernel extraction was stopped in 2005 due to costs and because the means appeared to be stable. The average number of plots sampled annually in the historical surveys (Tab. S2 in Supplementary material) ranged from 18 (Northern Plateau) to 44 (Central Range).

Recent surveys

In the 2012-2013 survey, motivated by the generalised distress of the cone processing industry, the INIA-CIFOR, in cooperation with the Forest Service of Valladolid, restarted the studies of nut and kernel yield and quality in the plots on the Northern Plateau. In subsequent years, the studies were extended again to Catalonia, the Central Range and South West Andalusia, where, as in the case of the N. Plateau, the presence of *L.* occidentalis has been confirmed (Fent & Kment 2011). In the case of the Northern Plateau the new sampling points were coincident with former INIA plots, which were still active. For the other regions, new sampling points were selected, aiming to cover the previously sampled areas and extending the network to new areas within the region. The average number of plots annually sampled in these recent surveys (Tab. S1 in Supplementary material) ranged from 5 (Central Range) to 34 (Northern Plateau). In these new sampling plots, cones were annually collected from five trees per plot, and a sample of 15 to 25 randomly selected healthy cones was sent to the laboratory for processing (see below).

Nut and kernel content analysis

The cones from a given plot and year were randomly separated into three to five sets (Tab. S2 in Supplementary material), including five cones each. Each set of cones was labelled, and its fresh weight was recorded using a scale with a precision of 1 g. Each set of cones was oven-dried at 45 °C for a minimum period of a week until the cones opened. After complete opening of the cones, the sample was weighed again (Tab. 1).

The pine nuts, still in the shell, were manually extracted from each set. Pine nuts shorter than 4 mm were considered as aborts and removed. The rest of the pine nuts were counted using an automatic seed counter, and weighed with a precision of 0.1 g. A subsample of 50 pine nuts (or all of them, if the whole sample was smaller) from each set was randomly selected for the analysis of kernel yield and weighed with a precision of 0.1 g. Pine nuts were cracked using a manual nut cracker and classified and counted as sound "Kernels" or as "Damaged" nuts. Similitude of damage with those observed in *L. occidentalis* experimental cages and feeding assays allowed us to use the damage classification established for the species (Calama et al. 2017b, Farinha et al. 2018a – Fig. S1 in Supplementary material). Sound kernel lots were weighed with a precision of 0.1 g.

From the above analysis we were able to

Tab. 2 - Mean values (± standard error) for kernel-per-cone yield, nut-per-cone yield and kernel-per-nut yield in the four regions in both historical and recent periods. P-value refers to statistical differences between periods for a given region. For the same row, the same lowercase indicates non-significant differences (p >0.05) between regions in the same period.

Yield	Period	Catalonia	SW Andalusia	Northern Plateau	Central Range
Kernel-per-cone	Historical	0.0236 ± 0.0006 [c]	0.0334 ± 0.0005 [b]	0.0352 ± 0.0006 [a]	0.0349 ± 0.0004 [a]
	Recent	0.0155 ± 0.0017 [b]	0.0321 ± 0.0020 [a]	0.0177 ± 0.0005 [b]	0.0299 ± 0.0013 [a]
	p-value	*0.0002*	*0.6028*	*<0.0001*	*0.0010*
Nut-per-cone	Historical	0.1379 ± 0.0024 [c]	0.1548 ± 0.0018 [b]	0.1387 ± 0.0017 [c]	0.1613 ± 0.0012 [a]
	Recent	0.1133 ± 0.0072 [b]	0.1604 ± 0.0061 [a]	0.1073 ± 0.0016 [b]	0.1532 ± 0.0044 [a]
	p-value	*0.0043*	*0.5488*	*<0.0001*	*0.1828*
Kernel-per-nut	Historical	0.1663 ± 0.0031 [c]	0.2165 ± 0.0020 [b]	0.2408 ± 0.0021 [a]	0.2126 ± 0.0012 [b]
	Recent	0.1229 ± 0.0108 [c]	0.1936 ± 0.0077 [a]	0.1529 ± 0.0026 [b]	0.1931 ± 0.0062 [a]
	p-value	*0.0001*	*0.0305*	*<0.0001*	*0.0020*

derive a series of variables characterizing nut content, yield and quality at set level (Tab. 1). Among these, the final kernel-per-cone yield (KCY) is the most important variable for industry, because it will define the amount of kernels yielded per kg of purchased cones, and is computed as the product of the intermediate nut-per-cone yield (NCY) and kernel-per-nut yield (KCY), thus KCY = NCY · KNY.

Methods

The hypothesis of a significant recent decline in nut and kernel yield in the four studied regions was tested by looking for significant differences between periods ("historical surveys", before 2006 *vs.* "recent surveys", since 2012) and between years in the variable kernel-per-cone yield, as well as in the intermediates nut-per-cone yield and kernel-per-nut yield. In addition, we evaluated the existence of inter-period differences in the other six variables (CFW*, NPC, NP_kg, WP, D%, WK – Tab. 1) characterizing nut and kernel output and quality. Additionally, we analysed the regional consistence of the decline by testing for interregional differences in the nine variables for the two analysed periods. Analyses were done using univariate ANOVAs, carried out separately for each region or period.

In a second step, we aimed to test whether there had been temporal changes in the influence exerted over the final kernel-per-cone yield by the two intermediate yield components, nut-per-cone yield and kernel-per-nut yield. Moreover, we also looked for temporal changes in the influence of the rest of covariates over the intermediate and final kernel yields. To carry out these analyses we computed Pearson's correlation coefficient among all variables separately for each region and period. In addition, we carried out a principal component analysis (PCA) for each period and region including all nine variables.

The third group of analyses were orientated towards disentangling the potential effects of climate and a potential biotic agent over the observed decline in kernel-per-cone yield and intermediate yields. Given the well-known relationship between annual rainfall and cone weight, and the influence of the cone weight on covariates affecting kernel-per-cone yield (number of pine nuts per cone and the rate of damaged nuts), we evaluated whether these relationships have changed over time. In particular, we checked for between-period differences in the pattern of relationship between these variables largely influencing nut yield and the standardized cone fresh weight (CFW*), by means of ANOVA's computed for each region and 100-g class of CFW*. Detection of significant differences between periods will give us evidence of other factors, apart from climate, controlling the rate of damaged nuts and number of pine nuts per cone.

Results

Spatial and temporal differences in nut and kernel yield

Our results evidenced a significant decrease in kernel-per-cone yield (kg of kernels per kg of fresh cones) in three out of the four regions analysed when comparing historical (before 2006) and current series of pine nut production (since 2012 – Tab. 2). In particular, KCY has decreased significantly in the Northern Plateau (from 0.035 to 0.018), Catalonia (0.024 to 0.015) and, to a lesser degree, in the Central Range (0.035 to 0.029). In the case of SW Andalusia, the slight decrease in KCY (0.033 to 0.032) was statistically nonsignificant.

As regards the two intermediate yields (Tab. 2), decreases in the nut-per-cone yield (kg of pine nuts in shell per kg cones) were only significant in Catalonia and the Northern Plateau (from 0.140 to 0.110, in both regions), but not in the Central Range or in SW Andalusia. In these two regions, NCY ranges from 0.150-0.160, significantly larger than in the Northern Plateau and Catalonia. On the other hand, the kernel-per-nut yield KNY (kg of kernel per kg of pine nuts in shell) decreased significantly in all four analysed regions. The reduction was more drastic in the Northern Plateau (from 0.241 to 0.153) than in Catalonia (0.166 to 0.123) or in the Central Range and SW Andalusia, where KNY only fell from approx. 0.210 to 0.190 in both cases. When testing interregional differences, the most notable finding is that while the Northern Plateau had been the region with the largest KNY value in the historical series, it is now only the third.

A more detailed annual analysis of the three yield variables (KCY, NCY and KNY) allowed us to identify whether the differences between periods are associated with a general trend, or rather, with extraordinary annual events. In relation to kernel-per-cone yield (Fig. 2), in the Northern Plateau we observed a significant decline in all the recent surveys compared with the historical ones. In Catalonia, the three recent series are among those with lower values, although we can identify similar values in some of the historical records. In the case of the Central Range and SW Andalusia, a recent survey (2014-2015 in both cases) revealed the lowest value for the whole series, while the other three recent surveys are close to the observed values in the historical series.

As for nut-per-cone yield (Fig. S2 in Supplementary material), the recent series for both the Central Range and SW Andalusia are quite similar to the historical series, while in Catalonia we observed a clearly decreasing pattern from the historical to the recent series. In the Northern Plateau it is possible to differentiate three different groups: one with values over 0.150, all included in surveys from the historical series, a second, with values between 0.100 and 0.135, in both historical and recent series, and a third, including three surveys (two from historical and one from the recent series) with NCY values below 0.100. The value for the 2014-2015 survey is worthy of note, with a NCY below 0.07, the lowest for the analysed series.

With respect to annual variability in kernel-per-nut yield, the most evident changes are detected in the Northern Plateau and Catalonia, where all the values in the recent series are significantly lower than all the years from the historical ones. In Catalonia, the historical series had shown KNY between 0.127 and 0.208 while the recent

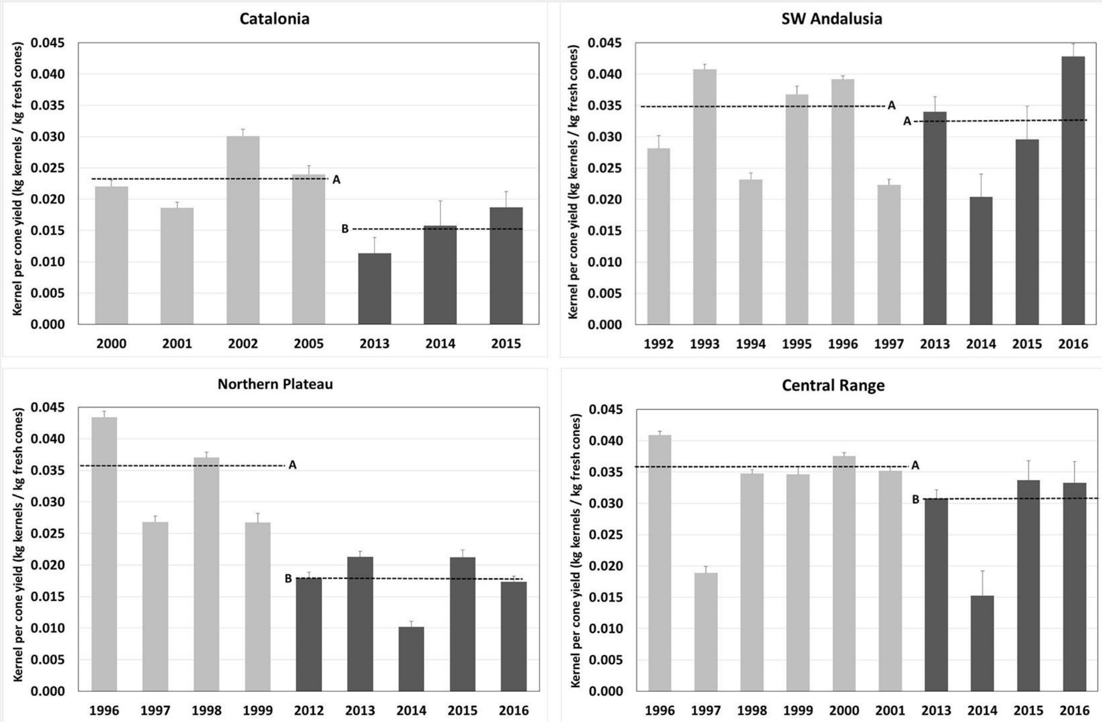

Fig. 2 - Interannual differences in Kernel per cone yield for each studied region. Light grey bars refers to historical series, dark grey bars to recent series. Whisker lines show standard error. Dashed line indicates average value for the period. Uppercase letter indicate differences between periods for the same region.

series ranges from 0.09 to 0.142, pointing to significant decreases. In contrast, in Andalusia and the Central Range, the observed slight but significant decrease between periods is mainly due to the effect of the exceptionally low value found in the 2014-2015 campaign (0.159 in SW Andalusia, 0.129 in Central Range), while the rest of the recent surveys were not statistically different from the historical series (Fig. S3 in Supplementary material).

Spatial and temporal differences in the covariates defining nut and kernel yield

As regards the evolution of the potential covariates explaining nut and kernel yield over time, the most notable finding was the highly significant increase observed in the rate of damaged or empty pine nuts (D – Tab. 3). Apart from SW Andalusia, with an increase of 37% (from 19% to 26%, p = 0.0414), in the other three regions we observed a 2- to 4-fold increment in D. The most significant increases were found in Catalonia and the Northern Plateau, where historical rates of damaged nuts were 23% and 13%, whereas more recent ones reached 56% and 49%, respectively. Furthermore, the rates of damaged pine nuts had never exceeded 20% (Northern Plateau) or 30% (Catalonia) for any of the years of the historical series, whilst in the recent series, the rates of damage always exceed 40% in both regions (Fig. 3). In the case of SW An-

Tab. 3 - Mean values (± standard error) for nut content variables in the four regions in both historical and recent periods. P-value refers to statistical differences in the subject variable between periods for a given region. For the same row, the same lowercase indicates non-significant differences (p >0.05) between regions in the same period. (CFW*): standardized fresh cone weight (g); (NPC): number of pine nuts per cone; (NP_kg): number of pine nuts per kg of fresh cones; (WP): weight of a single pine nut in shell (g); (D): rate of damaged or empty pine nuts; (WK): weight of a single kernel (g).

Variable	Period	Catalonia	SW Andalusia	Northern Plateau	Central Range
CFW*	Historical	257.8 ± 5.0 ^{c}	149.7 ± 2.4 ^{d}	269.5 ± 3.5 ^{b}	308.7 ± 2.6 ^{a}
	Recent	261.8 ± 12.0 ^{a}	245.9 ± 10.4 ^{ab}	229.1 ± 3.0 ^{b}	251.9 ± 7.0 ^{a}
	p-value	*0.8153*	*<0.0001*	*<0.0001*	*<0.0001*
NPC	Historical	65.6 ± 1.5 ^{d}	44.7 ± 0.7 ^{c}	76.1 ± 1.1 ^{b}	83.8 ± 0.8 ^{a}
	Recent	52.9 ± 3.6 ^{c}	65.5 ± 3.4 ^{b}	55.5 ± 1.0 ^{c}	80.1 ± 2.6 ^{a}
	p-value	*0.0138*	*<0.0001*	*<0.0001*	*0.3352*
NP_kg	Historical	270.4 ± 6.2 ^{b}	367.9 ± 9.4 ^{a}	292.8 ± 4.6 ^{b}	276.0 ± 2.5 ^{b}
	Recent	203.6 ± 11.1 ^{c}	266.7 ± 10.1 ^{b}	246.0 ± 4.3 ^{b}	328.0 ± 12.8 ^{a}
	p-value	*0.0019*	*0.0379*	*<0.0001*	*<0.0001*
WP	Historical	0.588 ± 0.012 ^{b}	0.537 ± 0.007 ^{c}	0.509 ± 0.005 ^{d}	0.615 ± 0.004 ^{a}
	Recent	0.575 ± 0.031 ^{a}	0.613 ± 0.019 ^{a}	0.455 ± 0.005 ^{b}	0.486 ± 0.013 ^{b}
	p-value	*0.7642*	*0.0236*	*<0.0001*	*<0.0001*
D	Historical	0.230 ± 0.012 ^{a}	0.186 ± 0.007 ^{b}	0.134 ± 0.007 ^{c}	0.094 ± 0.004 ^{d}
	Recent	0.560 ± 0.045 ^{a}	0.257 ± 0.037 ^{b}	0.492 ± 0.010 ^{a}	0.285 ± 0.024 ^{b}
	p-value	*<0.0001*	*0.0414*	*<0.0001*	*<0.0001*
WK	Historical	0.131 ± 0.003 ^{d}	0.139 ± 0.002 ^{c}	0.145 ± 0.002 ^{b}	0.154 ± 0.001 ^{a}
	Recent	0.186 ± 0.005 ^{a}	0.171 ± 0.005 ^{a}	0.152 ± 0.002 ^{b}	0.142 ± 0.004 ^{b}
	p-value	*<0.0001*	*0.0011*	*0.0077*	*0.0194*

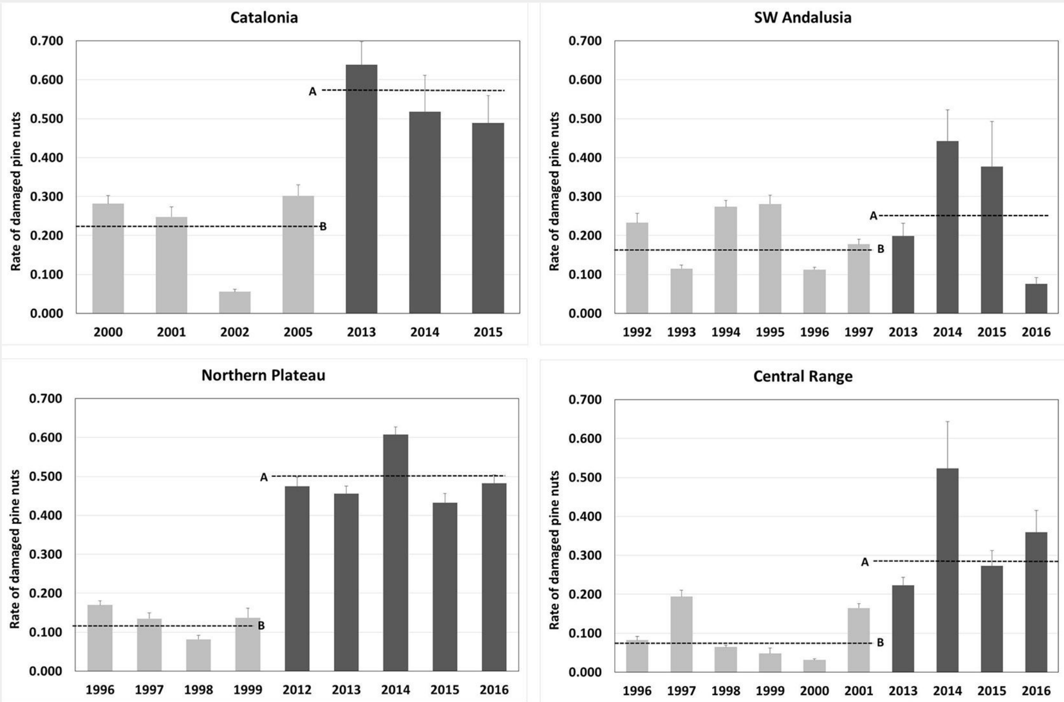

Fig. 3 - Interannual differences in rate of damaged pine nuts for each studied region. Light grey bars refers to historical campaigns, dark grey bar to recent campaigns. Whisker lines show standard error. Dashed line indicates average value for the period. Uppercase letter indicate differences between periods for the same region.

dalusia and the Central Range, the mean increment observed after 2012 is mainly due to two surveys with more than 30% damaged nuts.

As for the other covariates, the results are not consistent among regions. In Catalonia and the Northern Plateau we observed a highly significant decrease in the number of pine nuts per cone and per kg of fresh cones, while in the other two regions there has either been a non-significant decrease, or even a significant increase in these covariates. As an example, the number of pine nuts per cone in the Northern Plateau dropped from 76 to 55, while in the Central Range the values remain almost constantly (above a value of 80 pine nuts per cone), and in Andalusia number of pine nuts per cone has increased. The mean weight of pine nuts in shell decreased significantly after 2012 in the Northern Plateau and the Central Range, with no significant differences in Catalonia, and there was even an increase in SW Andalusia. These results are linked to similar trends observed in cone fresh weight.

Regionalised PCA confirm these patterns of shift between historical and recent series (Fig. 4 – only results for the Northern Plateau are shown). In the historical surveys, kernel-per-cone yield and nut-per-cone yield were highly correlated, and the number of pine nuts per cone was highly related with both, the three variables defining the first axis of variability, while the second axis was characterized by the negative correlation between the rate of damaged pine nuts with respect to kernel-per-nut yield and mean weights of cones, pine nuts and kernels. In contrast, in the recent campaigns the first axis defined the close relationship between the three components of yield (NCY, KNY and KCY) and their negative relationship with respect to rate of damaged pine nuts. In this case, Euclidean distance between kernel-per-nut yield and kernel-per-cone yield is much lower than in the historical records, pointing to an increased influence of kernel-per-nut yield and rate of damaged pine nuts over the final kernel-per-cone yield.

Temporal shift in the correlation among kernel-per-cone yield, intermediate yields and influential covariates

The influence of the different components of yield over final kernel-per-cone yield has varied through time in each region (Tab. 4). The most relevant finding is the generalized increase in the influence of kernel-per-nut yield over kernel-per-cone yield throughout the four studied regions. The coefficient for the correlation among KNY and KCY increases from 0.38-0.76 in the historical series to 0.77-0.91 in the recent series, while the coefficient for the correlation among NCY and KCY, which was the highest in the historical period for all regions (except in Catalonia), varied only slightly between periods. The correlation between NCY and KNY moved from

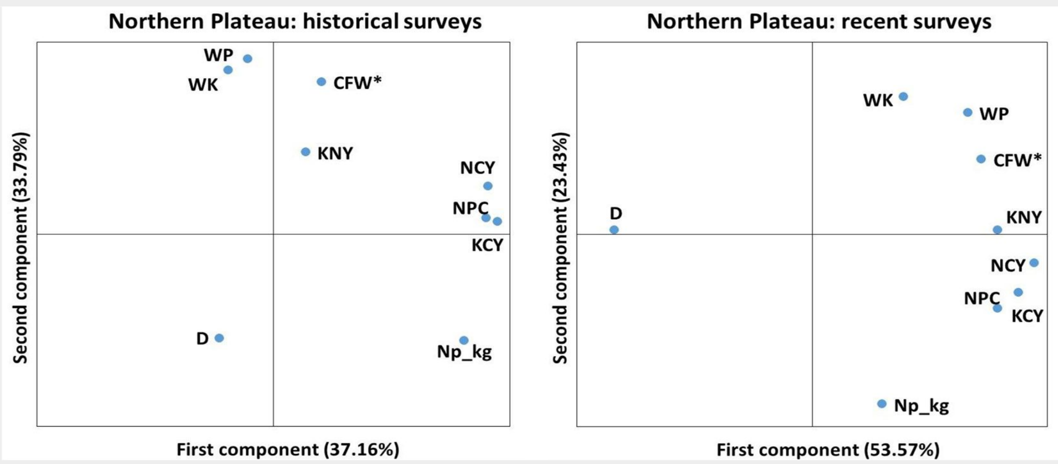

Fig. 4 - Principal component analysis plots over the two first axis, for the Northern Plateau historical (left) and recent (right) campaigns.

non-significant in the historical series (except in Catalonia) to significant in the recent series for all regions.

Tab. 5 shows how the influence exerted by the different analysed covariates over NCY, KNY and KCY have changed over time and regions. The most consistent result is that the number of pine nuts per cone is the covariate showing the highest correlation with NCY in all regions and for both periods, even increasing between periods. In addition, the importance of the rate of damaged nuts, the weight of a single pine nut, and the fresh cone weight in the NCY has also substantially increased, except in the Central Range.

Concerning kernel-per-nut yield, while in historical surveys the most correlated covariate was the mean kernel weight WK, in the recent surveys the highest (negative) correlation was observed with the rate of

Tab. 4 - Correlation coefficient between the different components of yield. Values below and over the diagonal for each region represents correlation values for the historical and recent series of nut production, respectively, for each region. (NCY): nut-per-cone yield; (KNY): kernel-per-nut yield; (KCY): kernel-per-cone yield. (*): p≤0.05; (ns): p>0.05.

Region	Components	NCY	KNY	KCY
Catalonia	NCY	-	0.587*	0.824*
	KNY	0.203*	-	0.909*
	KCY	0.745*	0.755*	-
Northern Plateau	NCY	-	0.508*	0.835*
	KNY	-0.013^{ns}	-	0.856*
	KCY	0.880*	0.409*	-
SW Andalusia	NCY	-	0.592*	0.915
	KNY	-0.052^{ns}	-	0.853*
	NCY	0.736*	0.573*	-
Central Range	NCY	-	0.269*	0.797*
	KNY	-0.097^{ns}	-	0.774*
	KCY	0.854*	0.383*	-

Tab. 5 - Temporal shift in the correlation coefficients relating nut contents covariates with the different components of nut yield at regional level. All the coefficients are significant ($p < 0.05$), except those indicated by "ns". ($): most influential covariate explaining nut-per-cone yield, kernel-per-nut yield and kernel-per-cone yield for each region and period; (CFW*): standardized fresh cone weight (g); (NPC): number of pine nuts per cone; (NP_kg): number of pine nuts per kg of fresh cones; (WP): weight of a single pine nut in shell (g); (D): rate of damaged or empty pine nuts; (WK): weight of a single kernel (g); (CAT): Catalonia; (SWA): South West Andalusia; (NP): Northern Plateau; (CR): Central Range.

Param	Kind	Nut-per-cone yield				Kernel-per-nut yield				Kernel-per-cone yield			
		CAT	SWA	NP	CR	CAT	SWA	NP	CR	CAT	SWA	NP	CR
CFW*	Historical	0.476	0.143	0.267	0.469	0.448	0.319	ns	ns	0.617	0.341	0.214	0.424
	Recent	0.596	0.513	0.557	ns	0.657	0.399	0.339	0.295	0.636	0.516	0.507	ns
Npc	Historical	0.695$^{\$}$	0.718$^{\$}$	0.801$^{\$}$	0.796$^{\$}$	ns	ns	ns	-0.097	0.440	0.560$^{\$}$	0.723$^{\$}$	0.698$^{\$}$
	Recent	0.874$^{\$}$	0.782$^{\$}$	0.873$^{\$}$	0.761$^{\$}$	0.501	0.430	0.378	0.460	0.736	0.693	0.701	0.756
WP	Historical	0.378	ns	0.133	0.317	0.597	0.399	0.103	ns	0.616	0.262	0.097	0.190
	Recent	0.581	0.492	0.483	ns	0.625	0.507	0.427	ns	0.589	0.569	0.502	ns
Np_kg	Historical	0.277	0.461	0.701	0.536	-0.465	-0.505	ns	ns	-0.128	ns	0.612	0.512
	Recent	0.562	0.559	0.574	0.640	ns	ns	ns	ns	0.385	0.422	0.346	0.508
D	Historical	-0.486	-0.197	-0.206	-0.496	-0.704	-0.586	-0.449$^{\$}$	-0.212	-0.711$^{\$}$	-0.553	-0.364	-0.507
	Recent	-0.640	-0.620	-0.591	-0.303	-0.969$^{\$}$	-0.934$^{\$}$	-0.952$^{\$}$	-0.946$^{\$}$	-0.935$^{\$}$	-0.847$^{\$}$	-0.884$^{\$}$	-0.775$^{\$}$
WK	Historical	0.165	-0.187	-0.097	-0.207	0.753$^{\$}$	0.627$^{\$}$	0.364	0.541$^{\$}$	0.601	0.196	0.087	0.094
	Recent	ns	ns	0.177	ns	ns	ns	0.262	ns	0.194	ns	0.241	ns

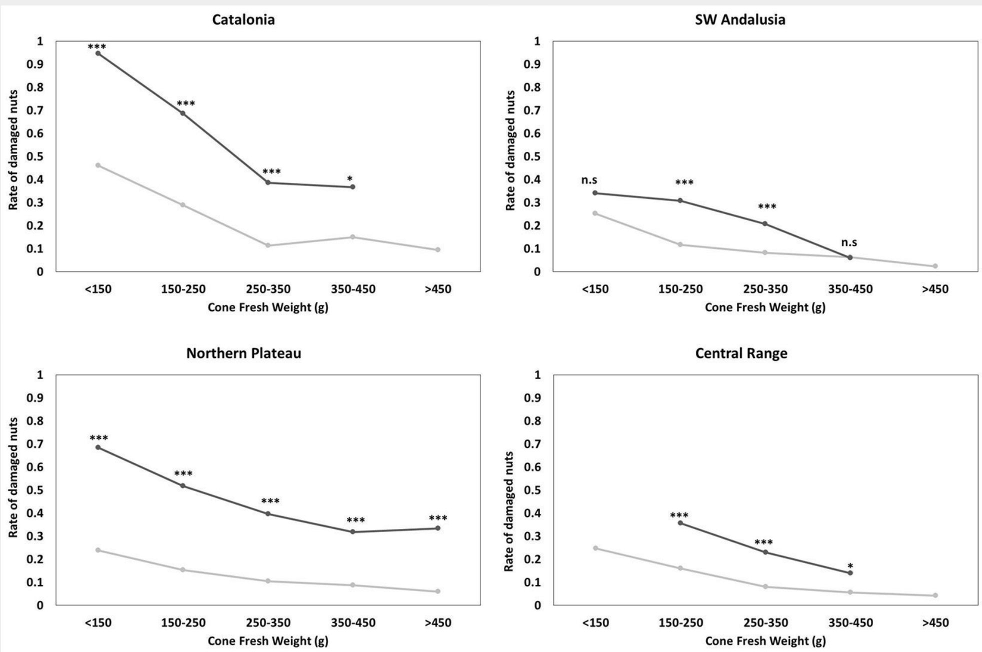

Fig. 5 - Influence of cone fresh weight on rate of damaged nuts. Black lines represents modern campaigns, while grey lines represents historical campaigns. (***, **, *): represents significant differences at p< 0.0001, p<0.01 and p<0.05 respectively, between periods for a given class of cone fresh weight. "n.s" indicates non-significant differences between periods for a given class of cone fresh weight.

damaged nuts for all regions, while correlation between WK and KNY is no longer significant. The number of pine nuts per cone was not significantly related with KNY in historical surveys, but is currently significantly related in all studied regions.

Finally, when focusing on the final kernel-per-cone yield, in historical surveys the number of pine nuts per cone was the most correlated covariate, except for Catalonia. In the recent surveys, the rate of damaged nuts is the most correlated covariate in all four regions, with correlation coefficient values ranging from -0.78 to -0.94, the number of pine nuts per cone now being the second most correlated (correlation coefficient from 0.69 to 0.76).

Influence of cone fresh weight on the covariates defining nut content

We observed a significant ($p < 0.05$) and negative relationship between cone fresh weight and rate of damaged pine nuts for both periods in all the regions. Moreover, we detected wide significant differences between periods for the 100-g classes of fresh cone weight in all the regions analysed, with consistently more damaged nuts after 2012 (Fig. 5), 30-50 % higher in Catalonia and the Northern Plateau and about 20 % higher in SW Andalusia and Central Range.

A positive correlation between mean cone weight and the number of pine nuts per cone persisted for both periods in all regions, with significant differences between periods only in the 150-250 g and 250-350 g classes of cone weight in the Northern Plateau and in the 150-250 g in Catalonia (with less pine nuts per cone than before 2006 – Fig. S4 in Supplementary material).

Discussion

The results presented in this work confirm and quantify for the first time the recent and generalized decline in the kernel-per-cone yield in *Pinus pinea* forests throughout Spain due to a sharp increase in the rate of damaged (or empty) pine nuts. Thanks to the extensive network of monitoring points and to the availability of data on pine nut and kernel yield from two series of surveys, one prior to 2006 and the other since 2012, it has been possible to confirm a statistically significant decrease in the final kernel-per-cone yield in three out of the four analysed regions, reaching values of 50% reduction in the Northern Plateau or 33% reduction in Catalonia, resulting in current KCY values over 0.015.

The observed magnitude of the decline in kernel-per-cone yield is in accordance with previous information reported for the distribution area of the species as a whole. Data from six processing industries in Portugal and Spain compiled by Mutke et al. (2017) showed a decrease in KCY from a stable 0.038 before 2008 to 0.022-0.028. Reports from Italy (Agri-Ciencia 2014) have indicated a reduction from 0.035 to 0.005, and severe reduction in KCY was also reported from Lebanon (Piqué et al. 2017) and Turkey (Parlak 2017). However, our results allow to confirm this decline at a much more detailed forest scale, over a wide variety of forests across the whole range for the species in Spain.

In addition, our findings allow to identify which of the intermediate yields is implied in the decline, providing insight into the potential causes for this reduction. In this regard, a significant decrease in nut-per-cone yield was only observed in the Northern Plateau and Catalonia, while for the kernel-per-nut yield, significant reductions were observed in all four regions. Before 2006, the nut-per-cone yield was the most important component affecting kernel-per-cone yield, while kernel-per-nut yield was almost constant (Calama et al. 2007, Morales 2009). From 2012 onwards, KNY exerts a greater influence on KCY, this being the main cause for the observed decrease in KCY. A final point to mention is that while both components tended to be unrelated and independent prior to 2006, after 2012 a significant positive correlation was found between them for all regions, pointing to the existence of a new factor influencing both intermediate contents in a similar way.

Since 2012, the rate of damaged pine nuts has significantly increased in the four studied regions, reaching mean values over 0.50 in Catalonia and the Northern Plateau and over 0.30 in SW Andalusia and Central Range, which equates closely with the increase in damaged pine nuts reported from different regions in the Mediterranean (Mutke et al. 2017, Farinha et al. 2017). The increased presence of normal-sized, but empty or damaged pine nuts is the main cause of the observed drop in the kernel-per-nut yield in *Pinus pinea*. Furthermore, the number of pine nuts per cone and the rate of damaged pine nuts has been correlated with the mean weight of cones (Morales 2009, Calama et al. 2007) and subsequently with the amount of rainfall during the last year of cone maturation (Mutke et al. 2007, Calama et al. 2016a, Guadaño et al. 2016); thus, we could infer a certain degree of climatic control over these two contents. However, our results show that for a given cone size, the rate of damaged pine nuts is much larger and the number of pine nuts per cone is lower in the recent surveys than in the historical ones (covering years with different climatic conditions in both periods), confirming that other factor, apart from climate, may control over the decline in kernel-per-cone yield.

Our results are in agreement with previous findings focusing on *L. occidentalis* (Farinha et al. 2017, 2018b, Elvira-Recuenco et al. 2016) as the main causal agent provoking the reduction in kernel-per-cone yield, based on feeding experiments in the field and laboratory-reared bugs. In addition, we have detected a higher number of seed abortions per cone (lower values of number of pine nuts per cone) coinciding with the years with larger rate of damaged pine nuts at least in two of the regions (Northern Plateau and Catalonia), which is in concordance with *L. occidentalis* damage on immature conelets reported for stone pine (Ponce et al. 2017, Farinha et al. 2018a) and other pine species (Strong et al. 2001, Lesieur et al. 2014).

However, our results do not exclude a possible second-order effect of climate, as suggested by Farinha et al. (2018a) or Elvira-Recuenco et al. (2016), who also detected a certain amount of damaged seeds in cones protected from *L. occidentalis* attack by means of exclusion bags. In addition, severe lack of precipitation outside the summer drought period, as occurred in the winter and spring seasons of 2017 in the Northern Plateau, resulted in very small cones (mean weight below 100 g). The survey in that year (results not shown) revealed the lowest kernel-per-cone yield of the whole series (with values below 0.005), associated with the highest rates of damaged nuts (over 0.87) and the lowest number of pine nuts per cone (13), evidencing that potential biotic damage can be aggravated by extreme dry years.

While there is a generalized increase in the rate of damaged pine nuts and the subsequent reduction in the kernel-per-cone yield throughout the four studied regions, we can identify different spatiotemporal patterns. The Northern Plateau and Catalonia show quite constant rates of damaged pine nuts, clearly exceeding the records from the historical series and pointing to a degree of stabilization and persistence in the damage. In addition, both regions show significant decrease in the number of pine nuts per cone. In contrast, in SW Andalusia and the Central Range, the damage in the recent series seems to be concentrated in one or two specific surveys, showing a descending trend in recent years. While spatial differences at within-region scale in the rate of damaged pine nuts have recently been associated with different aspects such as site productivity (Farinha et al. 2018b), stand stocking (Calama et al. 2017b) or forest composition (Villaseñor 2018), it is not clear which mechanisms could regulate interregional differences. The wide phenological differences between the regions, resulting in a two-month anticipated cone and seed maturation in SW Andalusia with respect to Northern Plateau and Catalonia (Montero et al. 2004), and the subsequent uncoupling with the lifecycle of *Leptoglossus* have been postulated as a possible explanation, though lacking of scientific evidences. As regards the temporal variability, higher rates of damage seem to occur in those years (*e.g.*, 2014-2015) with lower cone production, reinforcing the hypothesis of a biotic agent provoking a higher predatory pressure in years with scarce resources.

Conclusions

This study confirms and quantifies the existence of a generalized decline in the kernel-per-cone yield in *Pinus pinea* forests located in different Spanish regions. For the first time, the real magnitude of the decline has been quantified, revealing reductions of up to 50% in the final kernel-per-cone yield in the most affected regions. The observed decline in kernel-per-cone yield is mainly related to the observed increase in the rate of damaged pine nuts, and to a lesser extent, to the decrease in the number of pine nuts per cone. While both symptoms can be associated with extreme climate events our results reinforce the hypothesis of the implication of an additional biotic agent (presumably the exotic western conifer seed bug *L. occidentalis*). In this regard, the confirmed presence of the seed bug in the studied regions and the similitude between seed damage observed in the field and in controlled experiments support this hypothesis. However, as no efficient methods for monitoring the size of the insect population were available, it is not possible to unequivocally confirm its role as causative agent. In this regard, ongoing research into the lifecycle, feeding habits and monitoring techniques of *L. occidentalis* (Barta 2016) will permit greater insight into the causality and spatio-temporal variability of the damage.

Acknowledgements

RC, JG, SM and MP designed the study, carried the analysis and wrote the main part of the manuscript. MC, GM and EG carried out laboratory measurements and database processing. MJA, MiP, RG and GM collected and provided field data, and reviewed the manuscript.

This work has been carried out under the financial and functional framework of the PROPINEA agreement CC-16-095, between INIA, ITACYL and Deputation of Valladolid; EG-17-042-C2.2 agreement between INIA and MAPA; National Projects RTA-2013-00011.C2.1 and AGL-2017-83828-C2.1; and the H2020_CSA project 774632-INCREdible. Authors wish to thank personnel from the Forest Services of Valladolid, Ávila, Junta de Andalucía and CTFC for their permanent support with the inventory, cone collection and maintenance of the experimental trials.

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Supplementary Material

Tab. S1 - Main ecological attributes of the four analysed regions.

Tab. S2 - Number of plots annually sampled and sets annually processed for the four regions in both historical and recent surveys.

Fig. S1 - Different typologies of damaged pine nuts and kernels.

Fig. S2 - Interannual differences in Nuts per Cone Yield for each studied region.

Fig. S3 - Interannual differences in Kernel per Nut Yield for each studied region.

Fig. S4 - Influence of cone fresh weight on number of pine nuts per cone.

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